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Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Basics of Flow Cytometry

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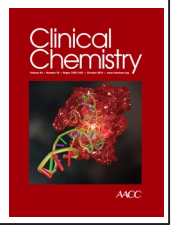

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Flow Cytometry

The measurement (-metry) of cells (cyto-) as they flow past a detection system.

The particles can be almost anything-cells, beads, bacteria, microvessicles, etc.

The characteristics being measured are referred to as parameters.

The History of Flow Cytometry

Mid-1950's

- Wallace Coulter patents and publishes his "Coulter Principle"

Mid-1960's to early 1970's

- Kamentsky at IBM's Watson Labs
- Fulwyler at Los Alamos National Laboratory

Late 1970's

- Becton-Dickinson, Coulter, and Ortho producing 4 parameter flow cytometers

Clinical Uses of Flow Cytometry

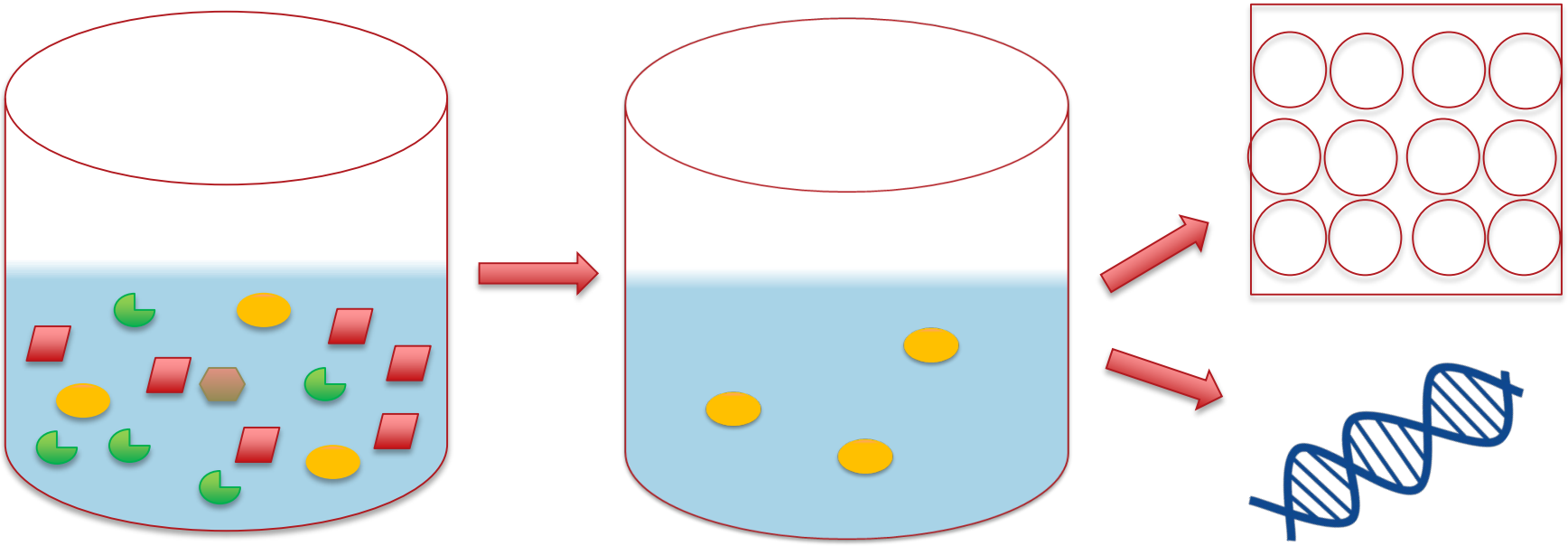
Current

- Hematology
- Oncology
- Immunology
- Blood Banking

Future

- Clinical research translates quickly into clinical diagnostics!

The Power of Flow Cytometry

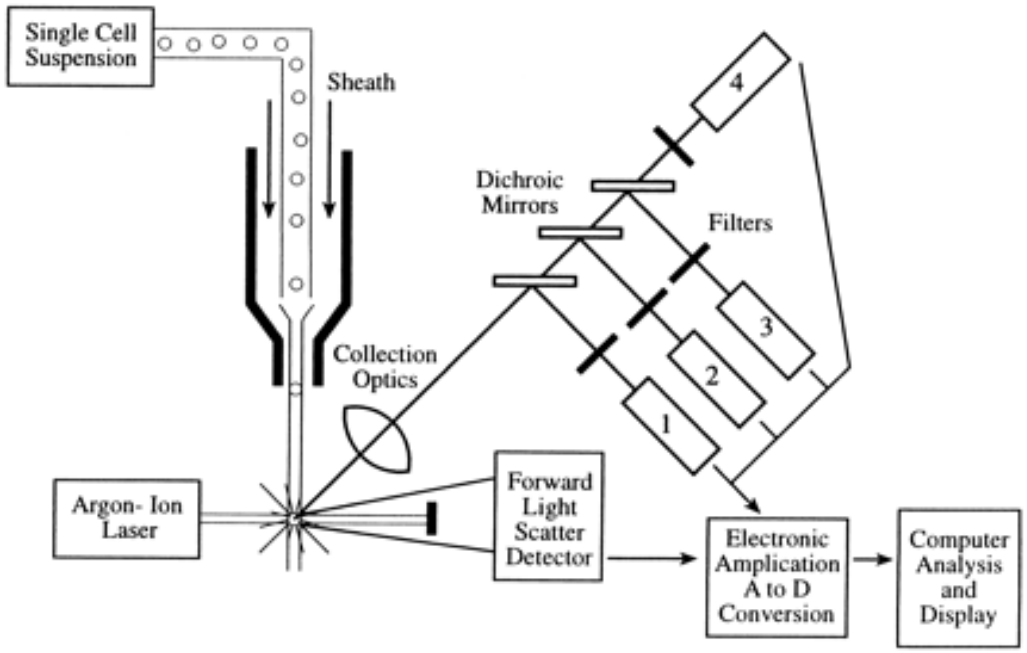


Determine Percentages and Absolute Counts of Specific Cell Types

Sort Specific Cell Types

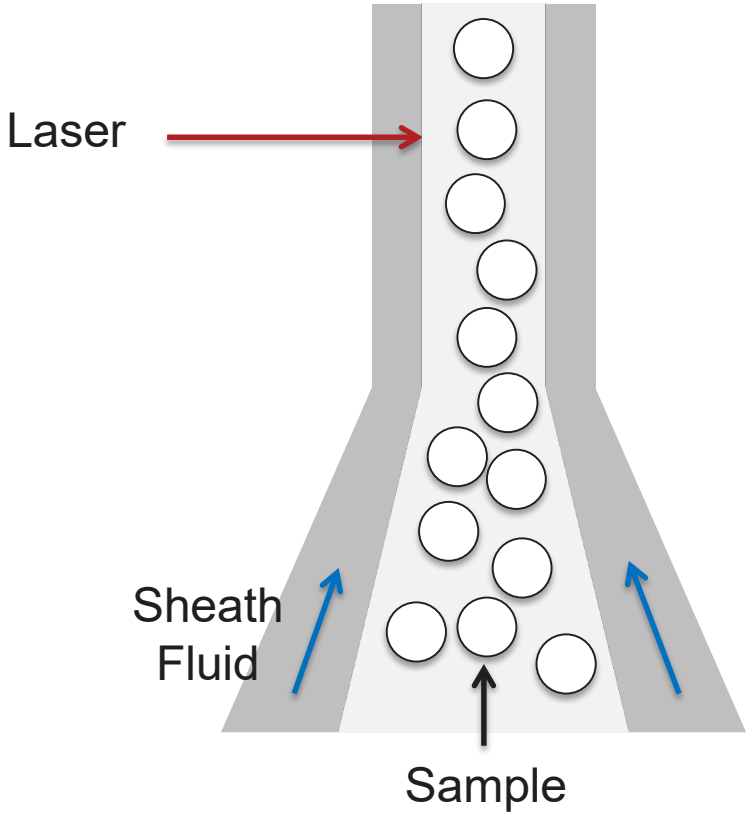
Perform Additional Analyses

Schematic of a flow cytometer



Brown M and Wittwer C. Flow Cytometry: Principles and Clinical Applications in Hematology. Clin Chem 2000; 46(8):1221-29.

Hydrodynamic Focusing



Optics


510/50
502LP
450/50
780/60
660/20
735LP
670LP
585/42
655LP
556LP
488/10
530/30
502LP
610LP
735LP
780/60

Electronics

Discriminator/Threshold

- Analyzed events must meet a certain minimum criteria

Photodiodes and Photomultiplier Tubes (PMTs)

- Paired with each filter and convert light into an electrical signal

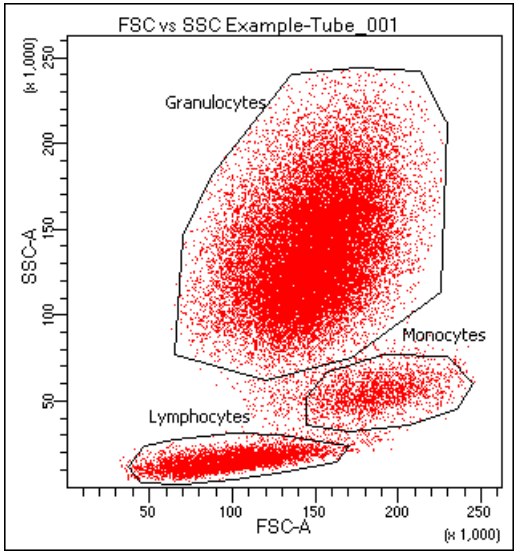
Analog to Digital Converter

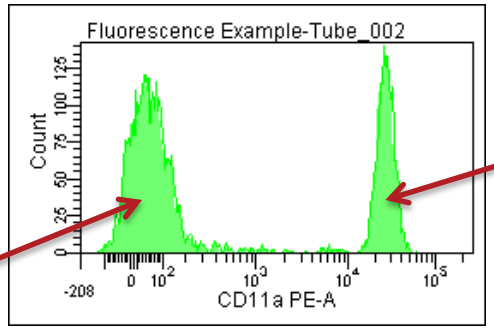
- Digital signals are then saved as a raw data file

Initiation of sorting by charging and deflecting particles

Parameters

- Intrinsic
  - Forward Scatter (FSC)
    - Small angle (0.5-5°)
    - Corresponds to cell size
  - Side Scatter (SSC)
    - Large angle (15-150°)
    - Corresponds to internal granularity, unevenness of the cell surface
- Extrinsic
  - Fluorescently-labeled (antibodies)
  - Dyes (nucleic acid, viability)





Positive Population

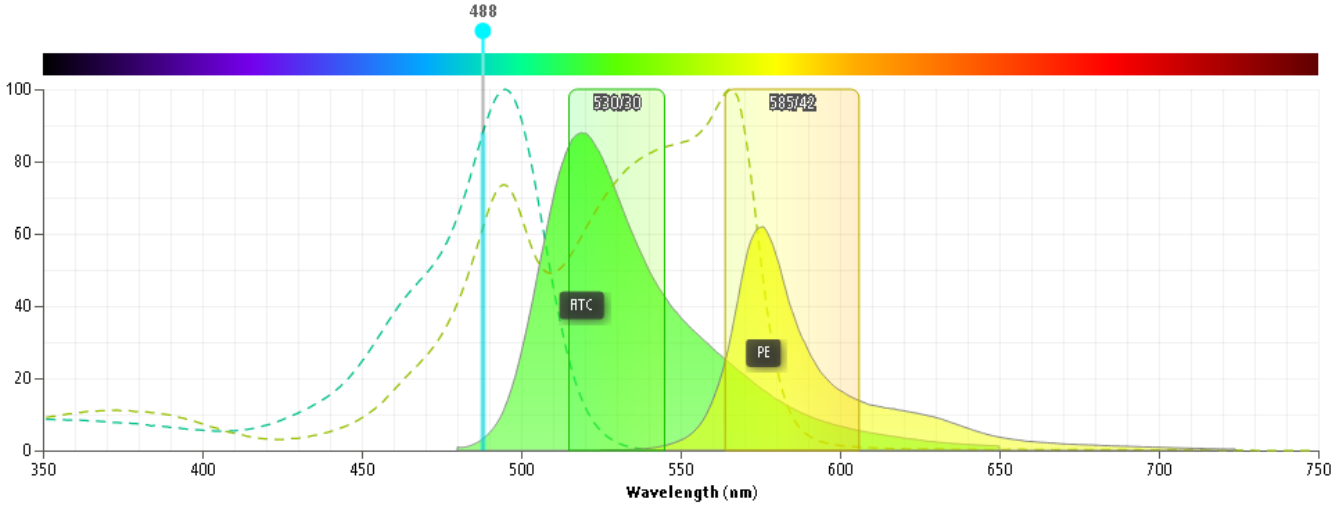
Negative Population

Instrument Settings

PMT Voltages

Auto-fluorescence

Spectral Overlap/Compensation



http://www.bdbiosciences.com/us/s/spectrumviewer?cc=US

Instrument Standardization

Increasingly important

- Complex assays
- Performed on multiple instruments within or between laboratories
- Patient results monitored over time

EuroFlow Consortium

http://www.euroflow.org/usr/pub/pub.php

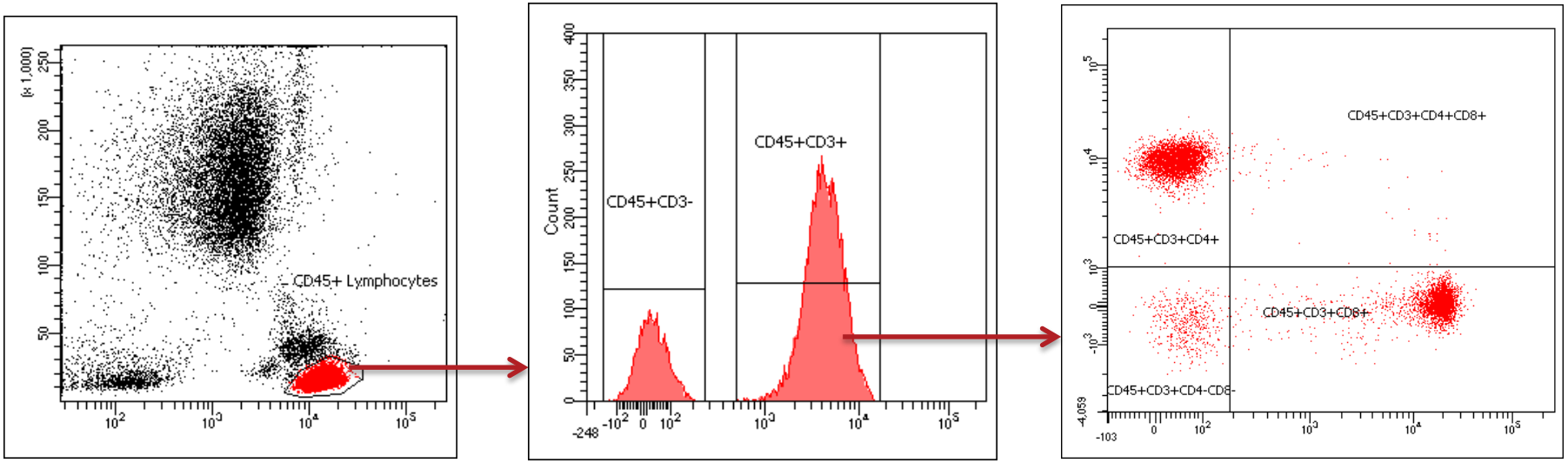
Data Analysis

Plots and Gates
- Histograms
- 2-parameter dot plots
- Multi-parameter plots

Gates
- Polygon
- Overlay
- Quadrant

Statistics
- Percentages
- Event numbers
- Mean fluorescence intensity (MFI)

Example Experiment



Population	#Events	%Parent	%Grand Parent
All Events	23,555	####	####
CD45+ Lymphocytes	9,945	####	####
CD45+CD3+	7,526	76.9	####
CD45+CD3+CD4+	4,086	54.3	41.7
CD45+CD3+CD4+CD8+	66	0.9	0.7
CD45+CD3+CD8+	2,889	38.4	29.5

Courses/Certifications

Multiple Courses Available from Many Different Sources

- Online/In Person
- Theory only/Wet Lab Hands-On

Flow Cytometry Certifications

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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- **Patents:** None Declared

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